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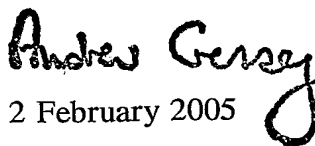
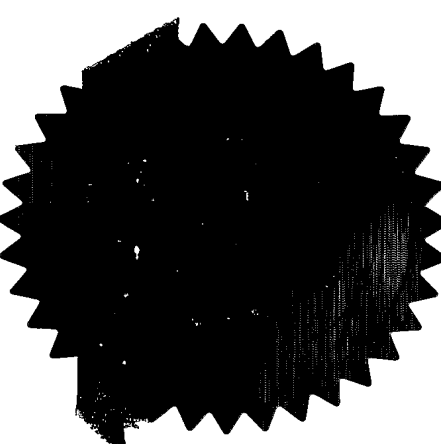
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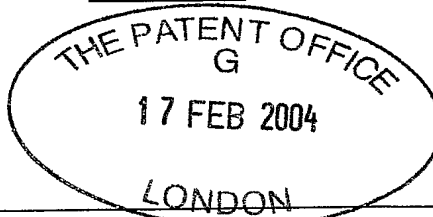
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1. Your reference

P10515TGB/JPD/51534.

17 FEB 2004

2. Patent application number

(The Patent Office will fill in this part)

0403509.3

3. Full name, address and postcode of the or of each applicant (underline all surnames)

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Patents ADP number (if you know it)

If the applicant is a corporate body, give the country/state of its incorporation

England and Wales

4. Title of the invention

Galanin Receptors and Brain Injury

5. Name of your agent (if you have one)

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

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Patents ADP number (if you know it)

1776001

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Country

Priority application number
(if you know it)

Date of filing
(day / month / year)

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application

Date of filing
(day / month / year)

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Any other documents (please specify) n/a

11. I/We request the grant of a patent on the basis of this application.

Signature *John Dean* Date 16th February 2004

12. Name and daytime telephone number of person to contact in the United Kingdom John Dean 0117 925 3030

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P105151GB

Galanin Receptors and Brain Injury

TECHNICAL FIELD

This invention relates to the field of protecting the central nervous system from injury, damage or disease.

The invention relates especially, but not exclusively, to protecting or treating the brain from the deleterious effects of (a) embolic, thrombotic or haemorrhagic stroke; (b) direct or indirect trauma to the brain or spinal cord; (c) surgery to the brain or spinal cord; (d) ischaemic or embolic damage to the brain resulting from cardiopulmonary bypass surgery, renal dialysis and reperfusion brain damage following myocardial infarction; (e) diseases of the brain that involve neuronal damage and/or cell death, such as Alzheimer's Disease, Parkinson's Disease, Multiple Sclerosis, vCJD (variant Creutzfeldt Jacob Disease); (f) immunological, chemical or radiation damage to the brain such as that caused by bacterial or viral infections, alcohol, chemotherapy for tumours and radiotherapy for tumours.

In particular, the invention relates to the use of ligands of galanin receptors, in particular, but not exclusively, the second galanin receptor subtype (GALR2), in the prevention or treatment of brain injury, damage or disease. The invention also relates to drug discovery methods for determining candidate drugs for use in the prevention or treatment of brain injury, damage or disease, and to pharmaceutical compositions for the prevention or treatment of brain injury, damage or disease.

BACKGROUND ART

Stroke

Stroke is defined as a cardiovascular accident, including an embolic, thrombotic or haemorrhagic episode that causes an area of brain anoxia, leading to permanent brain damage with associated functional neurological impairment. There are no satisfactory treatments for the neurological effects, despite stroke being the third-largest cause of death in the Western world. Stroke is responsible for much of the physical disability observed in the elderly population and up to 30% of stroke patients require long-term assistance with

daily activities. The number of strokes occurring annually in the US has been estimated at over 700,000 and in the UK, at any one time, 500,000 people have had a stroke at some time in their life. A number of neuroprotective agents have been developed to attempt to minimise the effects of a stroke but these have so far been disappointing in practice and are not in widespread or regular clinical use. These include, but are not limited to, the calcium channel antagonists nilvadipine (Nivadil®) from Fujisawa and nimodipine (Nimotop®) from Bayer; the antioxidants tirilazad (Freedox®) from Pharmacia & Upjohn and citicoline (CerAxon®) from Interneuron; and the protein kinase inhibitor fasudil (EriL™) from Asahi. In addition to calcium channel antagonists and free-radical scavengers, neuroprotective agents in development include N-methyl-D-aspartate (NMDA) antagonists, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) antagonists and other compounds designed to inhibit release of toxic neurotransmitters such as glutamate and glycine agonists.

Forms of traumatic or surgical brain injury

A range of conditions exist, other than stroke, in which brain damage occurs. These include direct or indirect trauma or surgery to the brain or spinal cord, surgery involving cardiopulmonary bypass, renal dialysis and reperfusion following myocardial infarction. The most common of these occurs during or after coronary artery bypass graft (CABG). 600,000 CABG surgeries are performed each year in the USA and 25% of all cardiopulmonary bypass patients exhibit neurological deficits within 3 months after surgery.

Diseases that damage the brain

Alzheimer's disease (AD) is a huge health burden in the Western world. AD is the commonest form of dementia in the elderly and there are currently an estimated 20 million people worldwide who have the disease. The incidence of AD is expected to double over the next 25 years as the population of elderly people increases. The annual cost of caring for AD sufferers in the UK is in excess of £5.5 billion. To date no known cure exists for the disease and few treatments (other than the acetylcholine esterase inhibitors) have been shown to substantially slow the progression of the disease.

Other diseases known to cause neuronal damage and/or cell death include Parkinson's Disease, Multiple Sclerosis and variant Creutzfeldt Jacob Disease.

Other forms of brain injury include immunological, chemical or radiation damage such as that caused by bacterial or viral infections, alcohol, chemotherapy for tumours and radiotherapy for tumours.

Galanin

The twenty-nine amino-acid neuropeptide galanin (Tatemoto *et al.* (1983) FEBS Lett. **164** 124-128) is widely expressed in both the central and peripheral nervous system and has strong inhibitory actions on synaptic transmission by reducing the release of a number of classical neurotransmitters (Fisone *et al.* (1987) Proc. Natl. Acad. Sci. USA **84** 7339-7343; Misane *et al.* (1998) Eur. J. Neurosci. **10** 1230-1240; Pieribone *et al.* (1995) Neurosci. **64** 861-876; Hokfelt *et al.* (1998) Ann. N.Y. Acad. Sci. **863** 252-263; Kinney *et al.* (1998) J. Neurosci. **18** 3489-3500; Zini *et al.* (1993) Eur. J. Pharmacol. **245** 1-7). These inhibitory actions result in a diverse range of physiological effects, including:

- a) an impairment of working memory (Mastropaolo *et al.* (1988) Proc. Natl. Acad. Sci. USA **85** 9841-9845) and long term potentiation (LTP, thought to be the electrophysiological correlate of memory) (Sakurai *et al.* (1996) Neurosci. Lett. **212** 21-24);
- b) a reduction in hippocampal excitability with a decreased predisposition to seizure activity (Mazarati *et al.* (1992) Brain Res. **589** 164-166); and
- c) a marked inhibition of nociceptive responses in the intact animal and after nerve injury (Wiesenfeld *et al.* (1992) Proc. Natl. Acad. Sci. USA **89** 3334-3337).

These neuromodulatory actions of galanin have long been regarded as the principal role played by the peptide in the nervous system. However, there is now a large body of evidence to indicate that injury to many of these neuronal systems markedly induces the expression of galanin at both the mRNA and peptide levels. Examples of such lesion studies include the up-regulation of galanin in:

- a) the dorsal root ganglion (DRG) following peripheral nerve axotomy (Hokfelt *et al.* (1987) Neurosci. Lett. **83** 217-220),

- b) magnocellular secretory neurons of the hypothalamus after hypophysectomy (Villar *et al.* (1990) *Neurosci.* **36** 181-199),
- c) the dorsal raphe and thalamus after removal of the frontoparietal cortex (decortication) (Cortes *et al.* (1990) *Proc. Natl. Acad. Sci. USA* **87** 7742-7746),
- d) the molecular layer of the hippocampus after an entorhinal cortex lesion (Harrison & Henderson (1999) *Neurosci. Lett.* **266** 41-44), and
- e) the medial septum (MS) and vertical limb diagonal-band (vdB) after a fimbria fornix bundle transection (Brecht *et al.* (1997) *Brain Res. Mol. Brain Res.* **48** 7-16).

These studies have led a number of investigators to speculate that galanin might play a cell survival or growth promoting role in addition to its classical neuromodulatory effects.

To test this hypothesis, transgenic animals were generated, bearing loss- or gain-of-function mutations in the galanin gene (Bacon *et al.* (2002) *Neuroreport* **13** 2129-2132; Holmes *et al.* (2000) *Proc. Natl. Acad. Sci. USA* **97** 11563-11568; Steiner *et al.* (2001) *Proc. Natl. Acad. Sci. USA* **98** 4184-4189; Blakeman *et al.* (2001) *Neuroreport* **12** 423-425). Phenotypic analysis of galanin knockout animals demonstrated that, surprisingly, the peptide acts as a survival factor to subsets of neurons in the developing peripheral and central nervous system (Holmes, 2000; O'Meara *et al.* (2000) *Proc. Natl. Acad. Sci. USA* **97** 11569-11574). Most recently, it has been demonstrated that this neuronal survival role is also relevant to the adult DRG. Sensory neurons are dependent upon galanin for neurite extension after injury, mediated by activation of the second galanin receptor subtype in a PKC-dependent manner (Mahoney *et al.* (2003) *J. Neurosci.* **23** 416-421). It was therefore hypothesised that galanin might also act in a similar manner in the central nervous system, reducing cell death in animal models of brain injury, damage or disease.

WO92/12997 discloses the sequence of human galanin. There is a discussion of studies by other workers involving the administration of rat galanin or its N-terminal fragments to augment the effect of morphine. This patent application suggests that galanin can be expected to exhibit analgesic effects such that it may be administered alone or in combination with other analgesics. The application claims the use of galanin or its

analogues in the treatment of pain and the use of galanin antagonists in the treatment of certain other conditions.

WO92/20709 discloses a number of putative galanin antagonists. The antagonists which are described are all based on the first 12 amino acids of galanin followed by partial sequences of other peptides i.e. chimeric peptides. Some may be agonists, some antagonists and some may be both depending on the receptor subtype. The application discloses that the antagonists may be useful for treatment of insulin-, growth hormone-, acetyl choline-, dopamine-, Substance P-, Somatostatin-, and noradrenaline-related conditions including Alzheimer's type dementia and intestinal disease, along with conditions in the fields of endocrinology, food intake, neurology and psychiatry. Such antagonists may also be useful as analgesics. The application discloses the results of studies using some of the antagonists described therein on various effects such as galanin inhibition of glucose stimulated insulin release; galanin induced inhibition of scopolamine induced acetylcholine (ACh) hippocampal release; galanin induced facilitation of the flexor reflex; the displacement of bound iodinated galanin in membrane binding studies. There is a suggestion in the application that the antagonists may be indicated for analgesia but there is no disclosure in the application of results to this effect. No positive or beneficial claims are made concerning the use of galanin agonists.

Ukai *et al.* (1995) Peptides **16** 1283-1286 describes an investigation into the effects of galanin on memory processes in mice. The results suggest that galanin impairs memory and other cognitive functions and that intermediate doses of galanin specifically elicit amnesia. No positive or beneficial claims are made concerning the use of galanin agonists.

JP-A-6172387 discloses a synthetic peptide and derivatives for effectively inhibiting the insulin-secretion suppressing action of galanin, expected to be useful as a galanin-antagonistic substance for the prevention and treatment of Alzheimer's Disease.

Bartfai *et al.* (1992) TiPS **13** 312-317 is a review article summarising the knowledge of the actions of galanin at that time and describing a series of high-affinity galanin antagonists.

The review indicates that galanin antagonists may be useful in the treatment of Alzheimer's Disease.

Wynick *et al.* (1993) *Nature* **364** 529-532 discusses the involvement of galanin in basal and oestrogen-stimulated lactotroph function and the release of the hormone prolactin.

WO92/15681 discloses a peptide having the amino acid sequence of human galanin and DNA clones encoding the peptide. The application suggests that galanin may play a role in pancreatic activity and claims methods of modulating pancreatic activity, or of stimulating the production of growth hormone, the methods involving the use of the disclosed peptides.

WO92/15015 discloses DNA encoding human galanin and methods for the identification of galanin antagonists.

WO97/26853 discloses isolated GALR2 (second galanin receptor subtype), cDNA encoding GALR2 and methods of identifying a chemical compound which specifically binds to GALR2. There is mention that GALR antagonists may be effective in the treatment of Alzheimer's Disease. There is no disclosure of methods of selecting a compound for use in a method of preventing or treating brain injury on the basis of whether or not a compound is a GALR2 agonist.

Crawley (1996) *Life Sci.* **58** 2185-2199 is a review article summarising the knowledge of the actions of galanin at that time. It indicates that centrally administered galanin produces deficits in learning and memory tasks in rats and that the use of galanin antagonists may be useful in the treatment of Alzheimer's Disease.

Liu *et al.* (1994) *J. Neurotrauma* **11** 73-82 describes the effect of intraventricular injection of galanin on the extent of traumatic brain injury (TBI) caused by central fluid percussion in rats and showed that galanin-treated rats had significantly less deficits in various sensory motor tasks. The paper attributes these effects to the neuromodulatory action of galanin, decreasing the release of excitatory amino acids such as glutamate. However, there was no

difference in a memory test (Morris water maze test) between galanin-treated and -untreated rats.

Luo *et al.* (1995) Neuropeptide **28** 161-166 is a study to examine the effects of acute section of the sciatic nerve on the excitability of the flexor reflex in decerebrate, spinalised, unanaesthetised rats, as a measure of the development of chronic pain states. It was found that galanin may be useful in inhibiting the pain response. There is no mention of the use of GALR2 agonists to prevent or treat brain damage, injury or disease.

EP-A-0918455 discloses that recovery from crush injury (indicative of the regenerative abilities of sensory axons in the sciatic nerve), neuron survival during development and long term potentiation (LTP) are reduced in mice lacking the galanin gene compared to wild-type mice. From these results, it was proposed that galanin agonists may be suitable for use in the preparation of medicaments for the repair of nerve damage. There is also mention that a galanin agonist is useful in the treatment of Alzheimer's Disease and associated memory loss. No mention was made of which galanin receptor subtype mediated these effects, nor the effects of galanin agonists in protecting the central nervous system from injury, damage or diseases other than Alzheimer's Disease.

In addition, the above patent application, along with EP-A-1342410, describes a mammal which has been engineered such that it lacks the galanin gene, particularly a mouse.

Galanin receptors

Three G-protein coupled galanin receptor subtypes have been identified, GALR1, GALR2 and GALR3 (Habert-Ortoli *et al.* (1994) Proc. Natl. Acad. Sci. USA **91** 9780-9783; Burgevin *et al.* (1995) J. Mol. Neurosci. **6** 33-41; Howard *et al.* (1997) FEBS Letts. **405** 285-290; Smith *et al.* (1997) J. Biol. Chem. **272** 24612-24616; Wang *et al.* (1997a) Mol. Pharmacol. **52** 337-343; Wang *et al.* (1997b) J. Biol. Chem. **272** 31949-31953; Ahmad *et al.* (1998) Ann. N.Y. Acad. Sci. **863** 108-119; Bloomquist *et al.* (1998) Biophys. Res. Commun. **243** 474-479; Kolakowski *et al.* (1998) J. Neurochem. **71** 2239-2251; Smith *et al.* (1998) J. Biol. Chem. **273** 23321-23326). Binding of galanin to GALR1 and GALR3 have been shown to inhibit adenylyl cyclase (Wang, 1998; Habert-Ortoli, 1994; Smith,

1998) by coupling to the inhibitory G_i protein. In contrast, activation of GALR2 stimulates phospholipase C and protein kinase C activity by coupling to $G_{q/11}$ (Fathi, 1997; Howard, 1997; Wang, 1997a; Wittau *et al.* (2000) *Oncogene* **19** 4199-4209).

The lack of receptor subtype-specific antisera and the paucity of galanin ligands that are receptor subtype-specific, continues to hamper the analysis of the functional roles played by each receptor. A major advance in the field has been the discovery that galanin 2-11 peptide (termed AR-M1896) preferentially binds to GALR2 with a 500-fold specificity compared to GALR1 and with an almost complete loss of GALR1 activation (Liu *et al.* (2001) *Proc. Natl. Acad. Sci. USA* **98** 9960-9964; Berger *et al.* (2004) *Endocrinology* **145** 500-507). There is no published data as to whether AR-M1896 binds, or activates, GALR3. AR-M1896 has previously been used to demonstrate that activation of GALR2 appears to be the principal mechanism by which galanin stimulates neurite outgrowth from adult sensory neurons of the peripheral nervous system (Mahoney, 2003). Galanin 1-15 peptide and galanin 1-16 peptide are also known to be portions of the full-length galanin neuropeptide which will activate a galanin receptor.

Throughout this specification, the term "GALR" indicates a receptor which is one of the group of receptors consisting of GALR1, GALR2 and GALR3. The group includes, without limitation, the human, rat and mouse receptors. The receptor may also be chimaeric in form (i.e. including GALR sequences from different species), truncated (i.e. shorter than a native GALR sequence) or extended (i.e. including additional sequence beyond that of a native GALR sequence). Activation of the receptor may be determined, for example, by an increase in intracellular calcium levels.

Throughout this specification, the term "GALR2 agonist" indicates a substance capable of triggering a response in a cell, as a result of the activation of GALR2 by the substance. Methods of identifying whether or not a compound is an agonist of a galanin receptor are known in the art, for example, Botella *et al.* (1995) *Gastroenterology* **108** 3-11 and Barblivien *et al.* (1995) *Neuroreport* **6** 1849-1852.

DISCLOSURE OF INVENTION

According to a first aspect of the invention, there is provided the use of a GALR2 agonist in the preparation of a medicament for the prevention or treatment of brain damage, injury or disease.

Advantageously, the use of a GALR2 agonist allows the prevention of brain damage, injury or disease, or an improvement in the condition of individuals who have suffered such brain damage, injury or disease, as a result of the ability of galanin and galanin agonists to reduce cell death in such situations. Galanin also acts as an endogenous neuroprotective factor to the hippocampus.

The brain injury or damage may be caused by one of: embolic, thrombotic or haemorrhagic stroke; direct or indirect trauma or surgery to the brain or spinal cord; ischaemic or embolic damage to the brain during cardiopulmonary bypass surgery or renal dialysis; reperfusion brain damage following myocardial infarction; brain disease; immunological damage, chemical damage or radiation damage. The immunological damage may be the result of bacterial or viral infection. The chemical damage may be the result of excess alcohol consumption or administration of chemotherapy agents for cancer treatment. The radiation damage may be the result of radiotherapy for cancer treatment.

The brain disease may preferably be one of Alzheimer's Disease, Parkinson's Disease, Multiple Sclerosis or variant Creutzfeld Jacob Disease.

The GALR2 agonist may be a polypeptide comprising a portion of the galanin amino acid sequence and preferably may be AR-M1896.

Alternatively, the GALR2 agonist may be a non-peptide small chemical entity.

The GALR2 agonist may have a binding affinity of between 0 and 100 μ M, preferably between 0 and 1 μ M.

According to a second aspect of the invention, there is provided a method for preventing or treating brain injury, damage or disease comprising administering an effective amount of a GALR2 agonist to an individual in need of such prevention or treatment.

The brain injury or damage may be caused by one of: embolic, thrombotic or haemorrhagic stroke; direct or indirect trauma or surgery to the brain or spinal cord; ischaemic or embolic damage to the brain during cardiopulmonary bypass surgery or renal dialysis; reperfusion brain damage following myocardial infarction; brain disease; immunological damage, chemical damage or radiation damage. The immunological damage may be the result of bacterial or viral infection. The chemical damage may be the result of excess alcohol consumption or administration of chemotherapy agents for cancer treatment. The radiation damage may be the result of radiotherapy for cancer treatment.

The brain disease may preferably be one of Alzheimer's Disease, Parkinson's Disease, Multiple Sclerosis or variant Creutzfeld Jacob Disease.

The GALR2 agonist may be a polypeptide comprising a portion of the galanin amino acid sequence and preferably may be AR-M1896.

Alternatively, the GALR2 agonist may be a non-peptide small chemical entity.

The GALR2 agonist may have a binding affinity of between 0 and 100 μ M, preferably between 0 and 1 μ M.

According to a third aspect of the invention, there is provided a method of selecting a candidate compound for use in a method for the prevention or treatment of brain injury, damage or repair, comprising determining whether at least one test compound is an agonist of GALR2 and selecting the at least one test compound as a candidate compound if it is an agonist of GALR2.

It may be determined that the at least one test compound binds to GALR2 with a binding affinity of between 0 and 100 μ M, preferably between 0 and 1 μ M.

The GALR2 may comprise at least a portion of human GALR2, or may be full-length human GALR2.

The GALR2 may comprise at least a portion of non-human GALR2, preferably rat or mouse GALR2, or may be full-length GALR2.

The GALR2 may be a chimeric receptor construct.

Using a method according to this aspect of the invention, a selection of test compounds may be screened in a high throughput screening assay.

According to a fourth aspect of the invention, there is provided a pharmaceutical composition for use in the prevention or treatment of brain injury, damage or disease, the composition comprising:

- a) an effective amount of at least one agonist of GALR2, or pharmaceutically acceptable salts thereof; and
- b) a pharmaceutically suitable adjuvant, carrier or vehicle.

The brain injury or damage may be caused by one of: embolic, thrombotic or haemorrhagic stroke; direct or indirect trauma or surgery to the brain or spinal cord; ischaemic or embolic damage to the brain during cardiopulmonary bypass surgery or renal dialysis; reperfusion brain damage following myocardial infarction; brain disease; immunological damage, chemical damage or radiation damage. The immunological damage may be the result of bacterial or viral infection. The chemical damage may be the result of excess alcohol consumption or administration of chemotherapy agents for cancer treatment. The radiation damage may be the result of radiotherapy for cancer treatment.

The brain disease may preferably be one of Alzheimer's Disease, Parkinson's Disease, Multiple Sclerosis or variant Creutzfeldt Jacob Disease.

The GALR2 agonist may be a polypeptide comprising a portion of the galanin amino acid sequence and preferably may be AR-M1896.

Alternatively the GALR2 agonist may be a non-peptide small chemical entity.

The GALR2 agonist may have a binding affinity of between 0 and 100 μ M, preferably between 0 and 1 μ M.

The pharmaceutically suitable adjuvant, carrier or vehicle may be selected from: ion exchangers, alumina, aluminium stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

The pharmaceutical composition may be administered orally or parenterally, preferably orally.

Where the pharmaceutical composition is administered orally, it may be in the form of a capsule or a tablet, and may preferably comprise lactose and/or corn starch. The pharmaceutical composition may further comprise a lubricating agent, preferably magnesium stearate. The pharmaceutical composition may be in the form of an aqueous suspension or aqueous solution, and may further comprise an emulsifying agent and/or a suspending agent. The pharmaceutical composition may comprise sweetening, flavouring and/or colouring agents.

The pharmaceutical composition may alternatively be administered by injection, by use of a needle-free device, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir.

Where the pharmaceutical composition is administered by injection or needle-free device, it may be in the form of a sterile injectable preparation or a form suitable for administration by needle-free device. The sterile injectable preparation or form suitable for administration by needle-free device may be an aqueous or an oleaginous suspension, or a suspension in a non-toxic parenterally-acceptable diluent or solvent. The aqueous suspension may be prepared in mannitol, water, Ringer's solution or isotonic sodium chloride solution. The oleaginous suspension may be prepared in a synthetic monoglyceride, a synthetic diglyceride, a fatty acid or a natural pharmaceutically-acceptable oil. The fatty acid may be an oleic acid or an oleic acid glyceride derivative. The natural pharmaceutically-acceptable oil may be an olive oil, a castor oil, or a polyoxyethylated olive oil or castor oil. The oleaginous suspension may contain a long-chain alcohol diluent or dispersant, preferably Ph. Helv.

Where the pharmaceutical composition is administered rectally, it may be in the form of a suppository for rectal administration. The suppository may comprise a non-irritating excipient which is solid at room temperature and liquid at rectal temperature. The non-irritating excipient may be one of cocoa butter, beeswax or a polyethylene glycol.

Where the pharmaceutical composition is administered topically, it may be an ointment comprising a carrier selected from mineral oil, liquid petroleum, white petroleum, propylene glycol, polyoxyethylene-polyoxypropylene compounds, emulsifying wax and water. Alternatively, it may be a lotion or cream comprising a carrier selected from mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

Where the pharmaceutical composition is administered nasally, it may be administered by nasal aerosol and/or inhalation.

BRIEF DESCRIPTION OF DRAWINGS

Embodiments of the invention will now be described, by way of example only, with reference to the accompanying Figures 1-3, in which:

Figure 1 shows the effects of intraperitoneal administration of 20mg/Kg kainate on hippocampal cell death *in vivo*;

Figure 2 shows the responses of galanin knockout, over expressing and wild-type hippocampal cultures after incubation with 10nM - 1 μ M staurosporine (St); and

Figure 3 shows the effect of co-administration of staurosporine or glutamate with galanin or AR-M1896 on galanin wild-type hippocampal cultures.

MODES OF CARRYING OUT THE INVENTION

Methods

Animals

All animals were fed standard chow and water *ad libitum*. Animal care and procedures were performed within the United Kingdom Home Office protocols and guidelines.

Galanin knockout mice

Details of the strain and breeding history have been published previously (Wynick *et al.* (1998) Proc. Natl. Acad. Sci. USA **95** 12671-12676). In brief, mice homozygous for a targeted mutation in the galanin gene were generated using the E14 cell line. A PGK-Neo cassette in reverse orientation was used to replace exons 1-5, and the mutation was bred to homozygosity and has remained inbred on the 129OlaHsd strain. Age and sex matched wild-type littermates were used as controls in all experiments.

Galanin over-expressing mice

Details of the strain and breeding history have been published previously (Bacon *et al.* (2002) *Neuroreport* 13 2129-2132). In brief, galanin over expressing mice were generated on the CBA/B6 F1 hybrid background. A mouse 129sv cosmid genomic library was screened and a ~25kb region was subcloned which contained the entire murine galanin coding region and ~20kb of upstream sequence. The transgene was excised by restriction digest and microinjected into fertilised oocytes at 5ng/ μ l final concentration. Four galanin over-expressing transgenic lines were generated as previously described (Bacon *et al.* (2002) *Neuroreport* 13 2129-2132) and galanin expression in the hippocampus was assessed by immunocytochemistry (see below). Line 46 was found to have highest levels of galanin expression in the CA1 and CA3 regions of the hippocampus, and in the dentate gyrus compared to the three other lines and wild-type controls. Line 46 was therefore used for all subsequent experiments.

Organotypic hippocampal cultures

Organotypic cultures were prepared as previously described (Elliott-Hunt *et al.* (2002) *J. Neurochem.* 80 416-425; Stoppini *et al.* (1991) *J. Neurosci. Methods* 37 173-182). Briefly, the hippocampi from 5-6 day old pups were rapidly removed under a dissection microscope and sectioned transversely at 400 μ m using a McIlwain tissue chopper (Mickle Laboratory Engineering Co. Ltd., Gomshall, UK). The slices were cultured in 95% air and 5% CO₂ at 37°C on a microporous transmembrane biopore membrane (Millipore, Poole, UK), in a 6-well plate, in 50% minimal essential medium with Earle's Salts (Gibco BRL, Paisley, UK) without L-glutamine, 50% Hanks Balanced Salt Solution (Gibco BRL), 25% Horse Serum (heat inactivated; Harlan Serum Labs, Loughborough, UK), 5mg/ml glucose (Sigma Chemical Co., Poole, UK) and 1ml glutamine (Sigma).

Preparation of primary neuronal cultures

Hippocampi from 2-3 day old pups were dissected and placed into 4°C collection buffer prepared with Hanks Balanced Salt Solution (calcium and magnesium free), (Gibco BRL, Paisley, UK), 10% (v/v) N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) (ICN Biomedicals Inc., Aurora, Ohio, USA), 50U/ml penicillin (Britannia Pharmaceuticals Ltd., Redhill, Surrey, UK), 0.05mg/ml streptomycin in 100ml (Sigma Chemical Company,

Poole, Dorset, UK), and 0.5% (v/v) Bovine Serum Albumin (BSA; ICN Biomedicals Inc., Aurora, Ohio, USA). Enzymatic digestion, isolation and culture of hippocampal neurons was performed as previously described (McManus & Brewer (1997) *Neurosci. Lett.* **224** 193-196). Cells were counted and plated at 40,000 cells/well onto D-L-poly-ornithine (Sigma) coated 96 well plates. After 24 hours 10 µg/ml 5'Fluoro 2' Deoxyuridine (Sigma; anti-mitotic agent) was added. Cultures were incubated at 37°C with ambient oxygen and 5% CO₂ for 9 days before experimentation. The media was changed after the first 3 days and then every fourth day thereafter.

Immunohistochemistry

Mice were intracardially perfused with 4% paraformaldehyde/Phosphate Buffered Saline (PBS). The brains were removed and post-fixed for 4 hours at room temperature. The brains were then equilibrated in 20% sucrose overnight at 4°C, embedded in Optimal Cutting Temperature (OCT) compound (Tissue Tek Ltd., Eastbourne, UK) mounting medium, frozen on dry ice, and cryostat-sectioned (30µm sections). Sections were blocked and permeabilised in 10% normal goat serum/PBS 0.2% Triton X-100 (PBST) for 1 hour at room temperature. Sections were then incubated in rabbit polyclonal antibody to galanin (Affinity, Nottingham, UK) at 1:1000 in PBST overnight at room temperature, washed 3 x 10 minutes in PBS, and incubated in fluorescein isothiocyanate (FITC)-goat (The Jackson Laboratory, Westgrove, PA, USA) at 1:800 for 3 hours at room temperature. After washing, sections were mounted in Vectashield™ (Vector Laboratories Inc., Burlington, CA, USA). Images were taken by using a Leica fluorescent microscope (Leica Microsystems, Milton Keynes, UK) with RT Color Spot camera and Spot Advance image capture system software (Diagnostic Instruments, Sterling Heights, MI, USA).

Galanin immunohistochemistry was also performed on dispersed hippocampal neurons and organotypic cultures which were fixed in 4% paraformaldehyde, permeabilised with Triton X-100 and then processed as above.

Staurosporine and glutamate induced hippocampal damage

Fourteen day organotypic hippocampal cultures were placed in 0.1% BSA with serum free media for 16 hours before incubation with varying concentrations of glutamic acid for 3

hours or staurosporine for 9 hours. Staurosporine and glutamate are both known to cause excitotoxic damage to such cell cultures (Prehn *et al.* (1997) *J. Neurochem.* **68** 1679-1685; Ohmori *et al.* (1996) *Brain Res.* **743** 109-115). Cultures were washed with serum-free medium and incubated for a further 24 hours before imaging. Regional patterns of neuronal injury in the organotypic cultures were observed by performing experiments in the presence of propidium iodide. After membrane injury, the dye enters cells, binds to nucleic acids, and accumulates, rendering the cell brightly fluorescent (Vornov *et al.* (1994) *Stroke* **25** 457-465). The CA1 neuronal subfield was clearly visible in a bright field image. Neuronal damage in the area encompassing the CA1 region was assessed using the density slice function in NIH Image software (Scion Image, MD, USA) to establish signal above background. The area of the subfields expressing the exclusion dye propidium iodide was measured, and expressed as a percentage of the total area of the subfields as assessed in the bright field image. Furthermore, for consistency in setting the parameters accurately when using the density slice function, the threshold was set against a positive control set of cultures exposed to 10mM glutamate.

Nine-day primary hippocampal cultures were exposed to staurosporine for 24 hours. The viability of neurons was measured by manual counting of both live and dead neurons using a live/dead kit (Molecular Probes, Lieden, Netherlands).

Treatments

Organotypic or dispersed primary hippocampal cultures were at various times cultured with or without the addition of the following chemicals: staurosporine (Sigma), L-glutamic acid (Sigma), galanin peptide (Bachem, Merseyside, UK), the high-affinity GALR2-specific agonist AR-M1896 [Gal(2-11)Trp-Thr-Leu-Asn-Ser-Ala-Gly-Tyr-Leu-Leu-NH₂] (AstraZeneca, Montreal, Quebec, Canada).

Kainate-induced hippocampal injury

8-week old female mice were injected with intraperitoneal (i.p.) kainic acid (Tocris Cookson, Bristol, UK) (20mg/kg) or vehicle (PBS, 1ml/kg). Kainic acid is known to cause hippocampal damage as previously described (Beer *et al.* (1998) *Brain Res.* **794** 255-266; Mazarati *et al.* (2000) *J. Neurosci.* **16** 6276-6281). Hippocampal cell death was measured

by terminal deoxynucleotidyl transferase-mediated fluorescein-dUTP nick end labelling (TUNEL). Animals were killed at 72 hours after injection with kainic acid or vehicle. Mice were intracardially perfused with 4% paraformaldehyde/PBS and the brains rapidly removed and post fixed for 4 hours at room temperature. The brains were equilibrated in 20% sucrose overnight at 4°C, embedded in OCT mounting media and frozen on dry ice. Sections were cut (16µm) on a cryostat, thaw mounted onto gelatine coated slides and stored at -80°C until use. Apoptosis was evaluated by using an *in situ* cell detection kit (Boehringer, Berkshire, UK). Every sixth section was collected and blocked with methanol and permeabilised with triton (0.1%) and sodium citrate (0.1%) and then labelled with fluorescein dUTP in a humid box for 1 hour at 37°C. The sections were then combined with horse radish peroxidase, colocalised with diaminobenzidine (DAB) and counterstained with haematoxylin. Controls received the same management except the labelling omission of fluorescein dUTP. After washing, sections were mounted in Vectashield™ (Vector Labs Inc.). Cells were visualised using a Leica fluorescent microscope with RT Colour Spot camera and Spot Advance image capture system software (Diagnostic Instruments Inc., Sterling Heights, MI, USA).

Statistical analysis

Data are presented as the mean + SEM. Student's t test was used to analyse the difference in staurosporine concentrations within groups. ANOVAs or non-parametric Mann-Whitney U post hoc tests were used as appropriate to analyse differences between genotypes and different ligands and/or staurosporine and glutamate points. A P value of <0.05 was considered to be significant.

Candidate compound screening method

Cells transfected with and stably expressing the cDNA encoding the human GALR2 were incubated with various compounds, thought to be putative GALR2 agonists, under conditions permitting binding of compounds to GALR2 (as described in WO97/26853). Activation of GALR2, indicating that the compound was an agonist of GALR2, was

detected by a variety of measures including an increase in intracellular calcium levels or change in intracellular pH.

Results

Experiment 1

Intraperitoneal administration of 20mg/kg kainic acid was used to induce excitotoxic hippocampal damage as previously described (Beer, 1998; Mazarati, 2000; Tooyama *et al.* (2002) *Epilepsia* **43 Suppl 9** 39-43). Three days later brains were harvested and hippocampal cell death assessed by counting the number of TUNEL-positive cells. The results are displayed in Figure 1. The number of apoptotic neurons was significantly greater in both the CA1 and CA3 regions of the galanin knockout animals (KO) compared to the strain-matched wild-type controls (WT) (Figure 1), an increase of 62.9% and 44.8% respectively (** $P < 0.01$, *** $p < 0.001$). Conversely, the degree of cell death was significantly lower in both the CA1 and CA3 regions of the galanin over-expressing animals (OE) than in strain matched controls (WT) (Figure 1), a decrease of 55.6% and 50.4% respectively ($p < 0.05$).

Experiment 2

To further dissect the neuroprotective role played by galanin in a more tractable *in vitro* system, both primary dispersed and organotypic hippocampal cultures (Elliott-Hunt, 2002) were used. These two techniques are complimentary since the dispersed hippocampal cultures ensure that observed effects are neuron-specific, whilst the organotypic cultures preserve the synaptic and anatomical organisation of the neuronal circuitry (Elliott-Hunt, 2002) as well as retaining many of the functional characteristics found *in vivo* (Adamschik *et al.* (2000) *Brain Res. Prot.* **5** 153-158). The effects of staurosporine and glutamate on neuronal cell death in hippocampal cultures (Prehn, 1997; Ohmori, 1996) were studied. Cell death was visualised by propidium iodide staining. Results are expressed as a percentage of the area expressing fluorescence as compared with the untreated "control" cultures. Staurosporine at 1 μ M and 100nM caused significant and consistent levels of neurotoxicity in both the wild-type (WT) and galanin knockout (KO) cultures. The percentage cell death was significantly higher in galanin knockout animals compared to

wild-type controls at both doses (1 μ M: $68 \pm 0.5\%$ vs $38 \pm 8\%$; 100nM: $65 \pm 10\%$ vs $40 \pm 26\%$; n=4, p<0.05), as shown in Figure 2A. Similarly, a marked and significant excess of cell death in the galanin knockout organotypic cultures after 9 hour exposure to 4mM glutamate was noted, compared to wild-type controls ($85 \pm 8.6\%$ vs $61 \pm 9.3\%$; n=4, p<0.05).

To ensure that the above effects were neuron-specific, the effects of staurosporine in dispersed primary hippocampal neurons were also studied. Once again a significant excess of cell death in the galanin knockout cultures was observed, compared to wild-type controls (n=4, p<0.01), over the range of 10nM - 1 μ M staurosporine (Figure 2B).

Experiment 3

Having demonstrated that an absence of galanin increases the susceptibility to hippocampal cell death, the studies were extended to the galanin over-expressing mice. A significant reduction in cell death was observed in the galanin over-expressing animals (OE) after exposure to 50nM or 100nM staurosporine, compared to strain-matched wild-type controls (WT) (Figure 2C; n=4, **p<0.01, ***p<0.001).

Experiment 4

To test whether exogenous galanin would protect wild-type hippocampal neurons from damage, 100mM galanin was co-administered with 100nM staurosporine to wild-type organotypic cultures. This co-administration provided significant neuroprotection (n=4, p<0.05) in these cultures (Figure 3A). Similarly, galanin was also protective over the dose range 10nM - 1 μ M when co-administered with 4mM glutamate in wild-type organotypic cultures (Figure 3B). In keeping with these findings using organotypic cultures, 100nM galanin also protected wild-type dispersed primary hippocampal neurons from cell death induced by 10nM staurosporine (Figure 3C; n=3, p<0.05).

Experiment 5

The neuroprotective effects of galanin in the hippocampus are likely to be mediated by activation of one or more of three G-protein coupled galanin receptor subtypes, GALR1, GALR2 and GALR3. It has previously been shown that activation of GALR2 appears to be

the principal mechanism by which galanin stimulates neurite outgrowth from adult sensory neurons (Mahoney, 2003). Therefore, the effect of 100nM AR-M1896 (a high-affinity GALR2-specific agonist), when co-administered with 100nM staurosporine in organotypic cultures from wildtype animals, was also tested. It should be noted that even if AR-M1896 does weakly activate GALR1, this would be most unlikely at 100nM when the IC_{50} for GALR1 is 879nM. AR-M1896 significantly reduced the amount of cell death in wild-type organotypic cultures to a similar amount observed with equimolar concentrations of galanin ($p < 0.05$, Figure 3A). The addition of AR-M1896 was also as effective in reducing staurosporine-induced cell death in galanin knockout cultures as that observed in the wild-type organotypic cultures (data not shown). Dispersed primary hippocampal neurons were also treated with AR-M1896 and staurosporine, demonstrating similar protective effects of the peptide to that observed with full-length galanin (Figure 3C). No significant effects of galanin or AR-M1896 were noted in the absence of staurosporine in organotypic or primary cultures.

SUMMARY

It has been demonstrated that galanin acts as an endogenous neuroprotective factor to the hippocampus, in a number of *in vivo* and *in vitro* models of injury. Further, exogenous galanin and a previously described high-affinity GALR2-specific agonist both reduced cell death. Therefore, GALR2 is the principal receptor subtype that mediates these protective effects. GALR2 signals by activation of PKC and hence the extracellular signal-regulated kinases (ERK) cascade (Wittau, 2000). The conclusion that GALR2 activation mediates the inhibition of neuronal cell death in hippocampal neurons, would be in keeping with existing studies that indicate that hippocampal protection is dependent upon activation of ERK (Maher (2001) *J. Neurosci.* **21** 2929-2938; Ozawa *et al.* (1999) *Neurosci. Lett.* **262** 57-60), and PkB/Akt (Culmsee *et al.* (2002) *Neurosci.* **115** 1089-1108; Gary *et al.* (2003) *J. Neurochem.* **84** 878-890). These data indicate that a GALR2-specific agonist may have therapeutic uses in the treatment or prevention of various forms of brain injury, damage or disease.

Claims

1. The use of a GALR2 agonist in the preparation of a medicament for the prevention or treatment of brain injury, damage or disease.
2. The use according to claim 1 wherein the brain injury or damage is caused by one of: embolic, thrombotic or haemorrhagic stroke; direct or indirect trauma or surgery to the brain or spinal cord; ischaemic or embolic damage to the brain during cardiopulmonary bypass surgery or renal dialysis; reperfusion brain damage following myocardial infarction; brain disease; immunological damage, chemical damage or radiation damage.
3. The use according to claim 2 wherein the immunological damage is the result of bacterial or viral infection.
4. The use according to claim 2 wherein the chemical damage is the result of excess alcohol consumption or administration of chemotherapy agents for cancer treatment.
5. The use according to claim 2 wherein the radiation damage is the result of radiotherapy.
6. The use according to claim 1 or 2 wherein the brain disease is one of Alzheimer's Disease, Parkinson's Disease, Multiple Sclerosis or variant Creutzfeld Jacob Disease.
7. The use according to any preceding claim wherein the GALR2 agonist is a polypeptide comprising a portion of the galanin amino acid sequence.
8. The use according to claim 7 wherein the GALR2 agonist is AR-M1896.
9. The use according to any of claims 1-6 wherein the GALR2 agonist is a non-peptide small chemical entity.
10. The use according to any preceding claim wherein the GALR2 agonist has a binding affinity of between 0 and 100 μ M.
11. The use according to claim 10 wherein the GALR2 agonist has a binding affinity of between 0 and 1 μ M.
12. A method for preventing or treating brain injury, damage or disease comprising administering an effective amount of a GALR2 agonist to an individual in need of such prevention or treatment.

13. A method according to claim 12 wherein the brain injury or damage is caused by one of: embolic, thrombotic or haemorrhagic stroke; direct or indirect trauma or surgery to the brain or spinal cord; ischaemic or embolic damage to the brain during cardiopulmonary bypass surgery or renal dialysis; reperfusion brain damage following myocardial infarction; brain disease; immunological damage, chemical damage or radiation damage.
14. A method according to claim 13 wherein the immunological damage is the result of bacterial or viral infection.
15. A method according to claim 13 wherein the chemical damage is the result of excess alcohol consumption or administration of chemotherapy agents for cancer treatment.
16. A method according to claim 13 wherein the radiation damage is the result of radiotherapy.
17. A method according to claim 12 or 13 wherein the brain disease is one of Alzheimer's Disease, Parkinson's Disease, Multiple Sclerosis or variant Creutzfeldt Jacob Disease.
18. A method according to any of claims 12-17 wherein the GALR2 agonist is a polypeptide comprising a portion of the galanin amino acid sequence.
19. A method according to claim 18 wherein the GALR2 agonist is AR-M1896.
20. A method according to any of claims 12-17 wherein the GALR2 agonist is a non-peptide small chemical entity.
21. A method according to any of claims 12-20 wherein the GALR2 agonist has a binding affinity of between 0 and 100 μ M.
22. A method according to claim 21 wherein the GALR2 agonist has a binding affinity of between 0 and 1 μ M.
23. A method of selecting a candidate compound for use in a method for the prevention or treatment of brain injury, damage or repair, comprising determining whether at least one test compound is an agonist of GALR2 and selecting the at least one test compound as a candidate compound if it is an agonist of GALR2.
24. A method according to claim 23 wherein it is determined that the at least one test compound binds to GALR2 with a binding affinity of between 0 and 100 μ M.
25. A method according to claim 24 wherein it is determined that the at least one test compound binds to GALR2 with a binding affinity of between 0 and 1 μ M.

26. A method according to any of claims 23-25 wherein the GALR2 comprises at least a portion of human GALR2.
27. A method according to claim 26 wherein the GALR2 is full-length human GALR2.
28. A method according to any of claims 23-25 wherein the GALR2 comprises at least a portion of non-human GALR2.
29. A method according to claim 28 wherein the GALR2 is rat or mouse GALR2.
30. A method according to claim 28 or 29 wherein the GALR2 is full-length GALR2.
31. A method according to any of claims 23-25 wherein the GALR2 is a chimeric receptor construct.
32. A method according to any of claims 23-31 wherein a selection of test compounds are screened in a high throughput screening assay.
33. A pharmaceutical composition for use in the prevention or treatment of brain injury, damage or disease, the composition comprising:
 - a) an effective amount of at least one agonist of GALR2, or pharmaceutically acceptable salts thereof; and
 - b) a pharmaceutically suitable adjuvant, carrier or vehicle.
34. A pharmaceutical composition according to claim 33 wherein the brain injury or damage is caused by one of: embolic, thrombotic or haemorrhagic stroke; direct or indirect trauma or surgery to the brain or spinal cord; ischaemic or embolic damage to the brain during cardiopulmonary bypass surgery or renal dialysis; reperfusion brain damage following myocardial infarction; brain disease; immunological damage, chemical damage or radiation damage.
35. A pharmaceutical composition according to claim 34 wherein the immunological damage is the result of bacterial or viral infection.
36. A pharmaceutical composition according to claim 34 wherein the chemical damage is the result of excess alcohol consumption or administration of chemotherapy agents for cancer treatment.
37. A pharmaceutical composition according to claim 34 wherein the radiation damage is the result of radiotherapy.
38. A pharmaceutical composition according to claim 33 or 34 wherein the brain disease is one of Alzheimer's Disease, Parkinson's Disease, Multiple Sclerosis or variant Creutzfeld Jacob Disease.

39. A pharmaceutical composition according to any of claims 33-38 wherein the GALR2 agonist is a polypeptide comprising a portion of the galanin amino acid sequence.
40. A pharmaceutical composition according to claim 39 wherein the GALR2 agonist is AR-M1896.
41. A pharmaceutical composition according to any of claims 33-38 wherein the GALR2 agonist is a non-peptide small chemical entity.
42. A pharmaceutical composition according to any of claims 33-41 wherein the GALR2 agonist has a binding affinity of between 0 and 100 μ M.
43. A pharmaceutical composition according to claim 42 wherein the GALR2 agonist has a binding affinity of between 0 and 1 μ M.
44. A pharmaceutical composition according to any of claims 33-43 wherein the pharmaceutically suitable adjuvant, carrier or vehicle is selected from: ion exchangers, alumina, aluminium stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.
45. A pharmaceutical composition according to any of claims 33-44 which is administered orally or parenterally.
46. A pharmaceutical composition according to claim 45 which is administered orally.
47. A pharmaceutical composition according to claim 46 which is in the form of a capsule or a tablet.
48. A pharmaceutical composition according to claim 47 which comprises lactose and/or corn starch.
49. A pharmaceutical composition according to claim 48, further comprising a lubricating agent.
50. A pharmaceutical composition according to claim 49 wherein the lubricating agent is magnesium stearate.

51. A pharmaceutical composition according to claim 47 which is in the form of an aqueous suspension or aqueous solution.
52. A pharmaceutical composition according to claim 51 which comprises an emulsifying agent and/or a suspending agent.
53. A pharmaceutical composition according to any of claims 46-52 which comprises sweetening, flavouring and/or colouring agents.
54. A pharmaceutical composition according to claim 45 which is administered by injection, by needle-free device, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir.
55. A pharmaceutical composition according to claim 54 which is administered by injection.
56. A pharmaceutical composition according to claim 55 which is in the form of a sterile injectable preparation.
57. A pharmaceutical composition according to claim 56 wherein the sterile injectable preparation is an aqueous or an oleaginous suspension, or a suspension in a non-toxic parenterally-acceptable diluent or solvent.
58. A pharmaceutical composition according to claim 54 which is administered by needle-free device.
59. A pharmaceutical composition according to claim 58 which is a form suitable for administration by needle-free device.
60. A pharmaceutical composition according to claim 59 wherein the form suitable for administration by needle-free device is an aqueous or an oleaginous suspension, or a suspension in a non-toxic parenterally-acceptable diluent or solvent.
61. A pharmaceutical composition according to claim 57 or 60 wherein the aqueous suspension is prepared in mannitol, water, Ringer's solution or isotonic sodium chloride solution.
62. A pharmaceutical composition according to claim 57 or 60 wherein the oleaginous suspension is prepared in a synthetic monoglyceride, a synthetic diglyceride, a fatty acid or a natural pharmaceutically-acceptable oil.
63. A pharmaceutical composition according to claim 62 wherein the fatty acid is an oleic acid or an oleic acid glyceride derivative.

64. A pharmaceutical composition according to claim 62 wherein the natural pharmaceutically-acceptable oil is an olive oil, a castor oil, or a polyoxyethylated olive oil or castor oil.
65. A pharmaceutical composition according to claim 62, 63 or 64 wherein the oleaginous suspension contains a long-chain alcohol diluent or dispersant.
66. A pharmaceutical composition according to claim 65 wherein the long-chain alcohol diluent or dispersant is Ph. Helv.
67. A pharmaceutical composition according to claim 54 which is administered rectally.
68. A pharmaceutical composition according to claim 67 which is in the form of a suppository for rectal administration.
69. A pharmaceutical composition according to claim 68 wherein the suppository comprises a non-irritating excipient which is solid at room temperature and liquid at rectal temperature.
70. A pharmaceutical composition according to claim 69 wherein the non-irritating excipient is one of cocoa butter, beeswax or a polyethylene glycol.
71. A pharmaceutical composition according to claim 54 which is administered topically.
72. A pharmaceutical composition according to claim 71 which is an ointment comprising a carrier selected from mineral oil, liquid petroleum, white petroleum, propylene glycol, polyoxyethylene-polyoxypropylene compounds, emulsifying wax and water.
73. A pharmaceutical composition according to claim 71 which is a lotion or cream comprising a carrier selected from mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.
74. A pharmaceutical composition according to claim 54 which is administered nasally.
75. A pharmaceutical composition according to claim 74 which is administered by nasal aerosol and/or inhalation.

ABSTRACT**Galanin Receptors and Brain Injury**

There is provided the use of a GALR2 agonist in the preparation of a medicament for the prevention or treatment of brain injury, damage or disease, wherein the brain injury or damage is caused by one of: embolic, thrombotic or haemorrhagic stroke; direct or indirect trauma or surgery to the brain or spinal cord; ischaemic or embolic damage to the brain during cardiopulmonary bypass surgery or renal dialysis; reperfusion brain damage following myocardial infarction; brain disease; chemical damage as the result of excess alcohol consumption or administration of chemotherapy agents for cancer treatment; radiation damage; or immunological damage as the result of bacterial or viral infection. The brain disease may be one of Alzheimer's Disease, Parkinson's Disease, Multiple Sclerosis or variant Creutzfeld Jacob Disease.

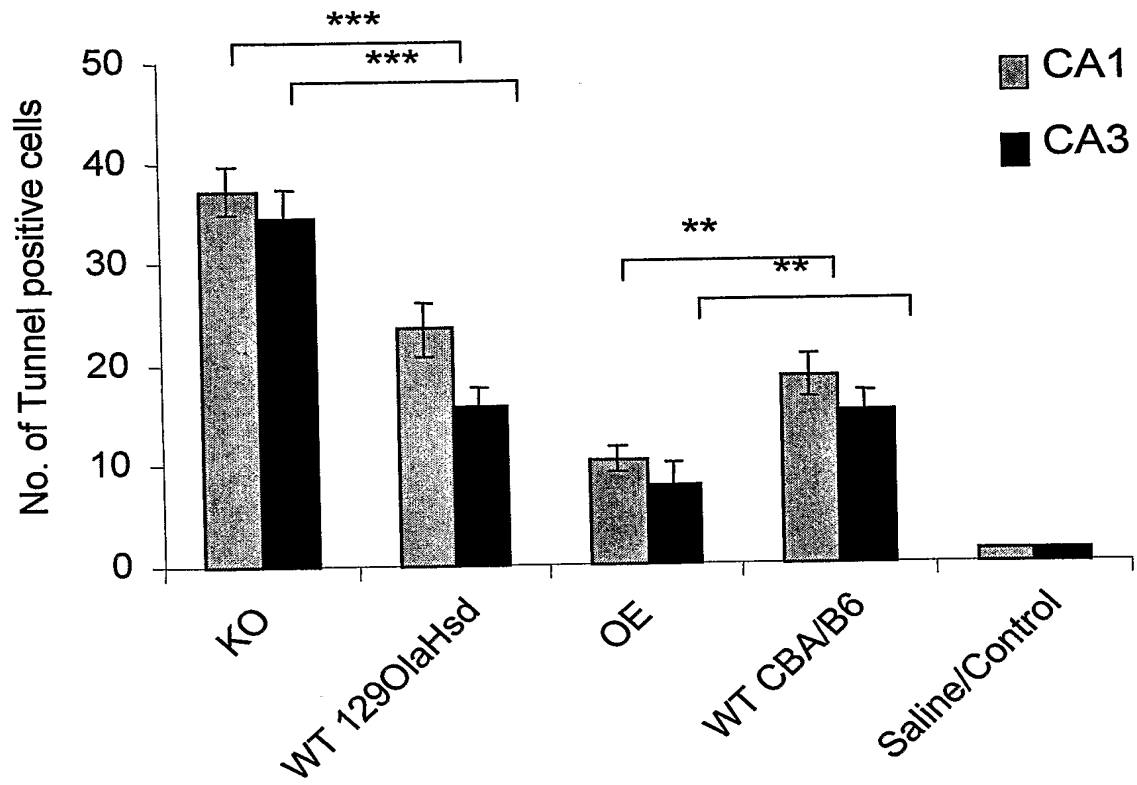
Figure 1



Figure 2

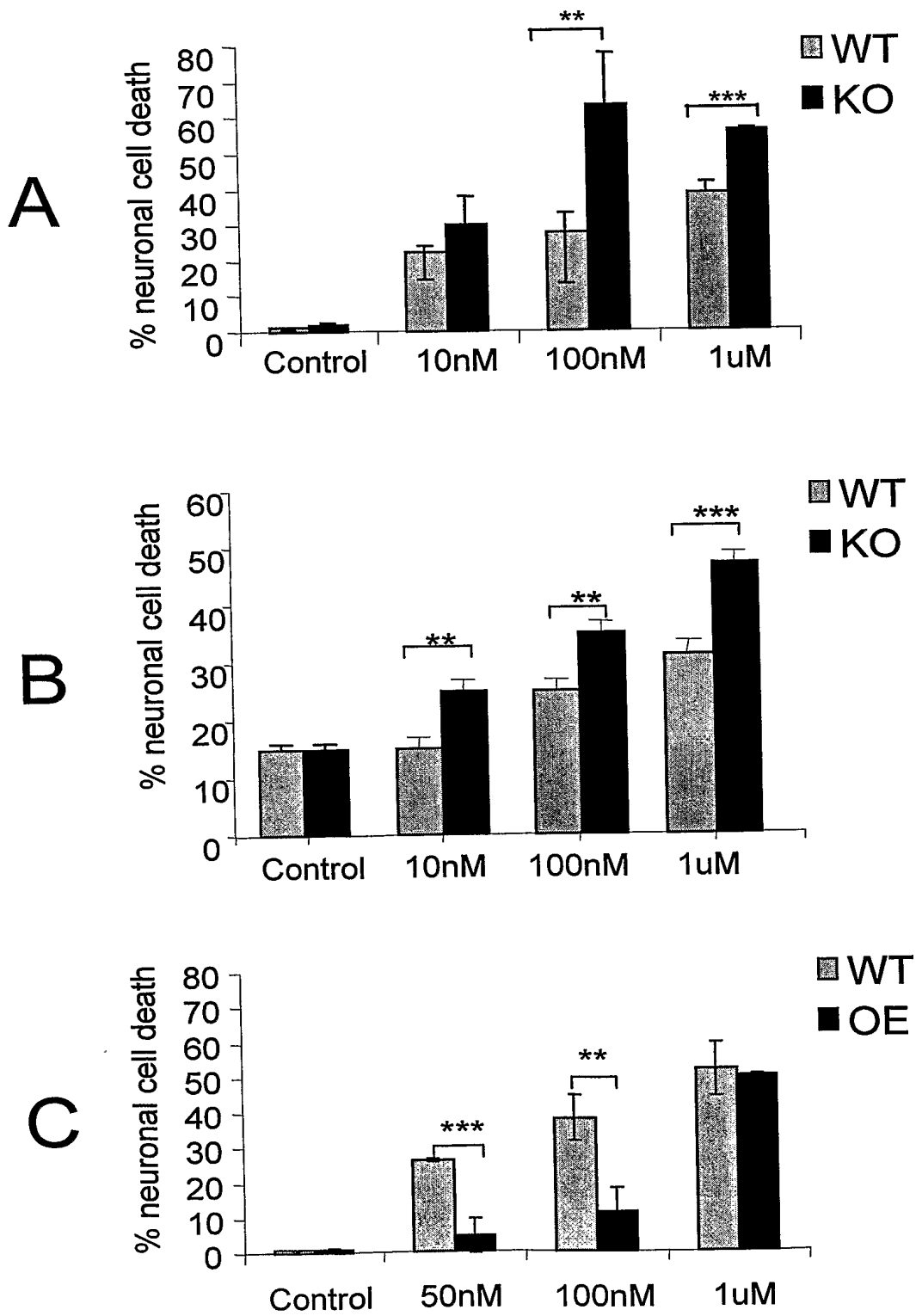
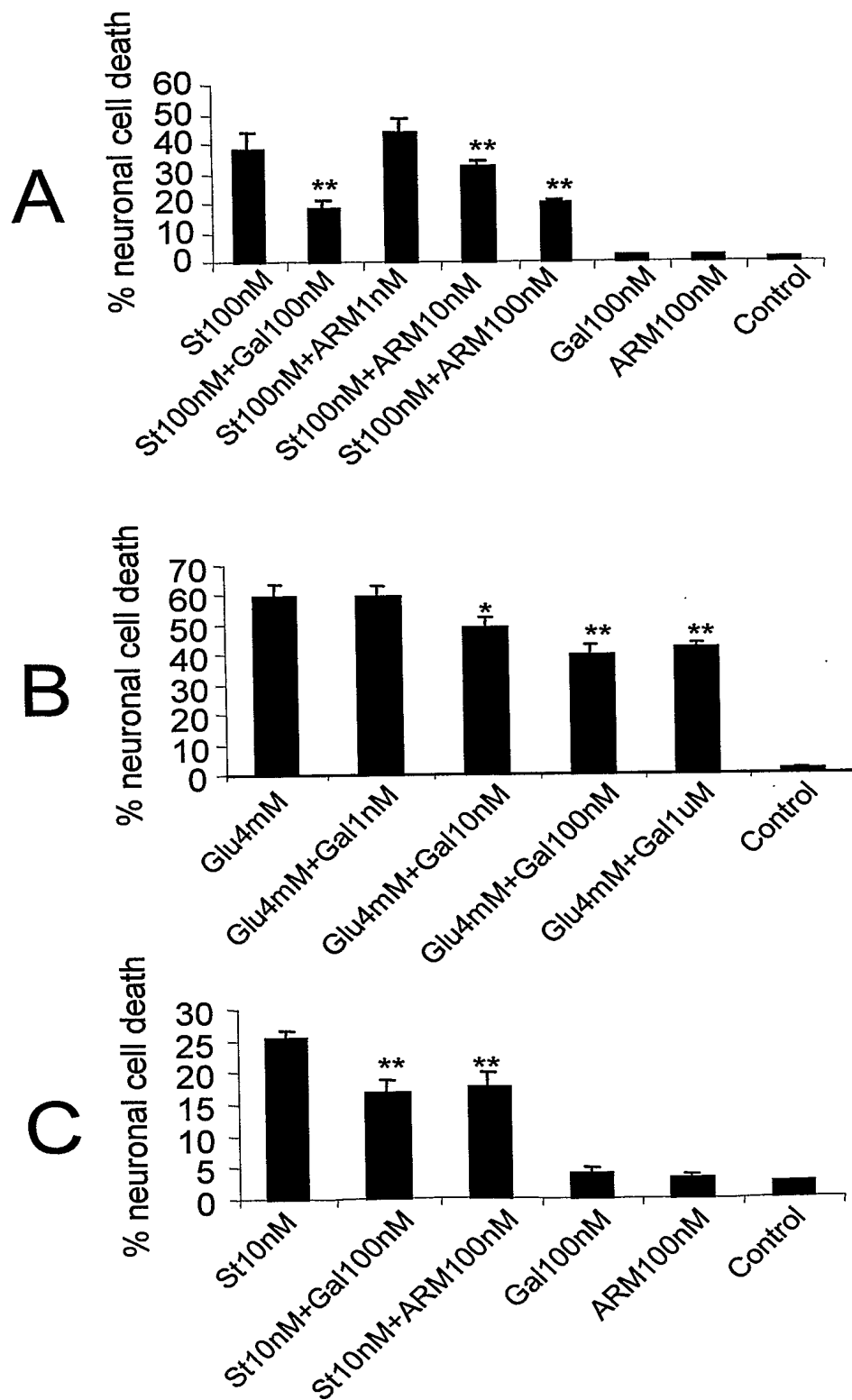




Figure 3



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